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Derivatives of 7-amino-1,2,3,4-tetrahydroisoquinoline and isophthalic acids as novel fibrinogen receptor antagonists

Olga L. Malovichko, Anna S. Petrus, Andrei A. Krysko,* Tatyana A. Kabanova, Sergei A. Andronati, Tamara L. Karaseva and Anna V. Kiriyak

Department of Medicinal Chemistry, A.V. Bogatsky Physico-Chemical Institute of the National Academy of Sciences of Ukraine, 86 Lustdorfskaya doroga, 65080 Odessa, Ukraine

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Abstract—The novel fibrinogen receptor antagonists containing fragments of 7-amino-1,2,3,4-tetrahydroisoquinoline and isophthalic acids were synthesized and successfully tested for their ability to inhibit platelet aggregation in vitro and to block FITC-Fg binding to $\alpha_{IIb}\beta_3$ on washed human platelets.

One of the main causes of myocardial infarction, stroke, peripheral ischemia, and paralysis is the process of thrombus formation.[1] Anticoagulants, fibrinolytics, and antiaggregants are used for the therapeutic treatment of thrombotic events. Fibrinogen receptor antagonists are the most interesting objects as antiaggregants.[2] Formation of the supramolecular complexes of fibrinogen with its receptor ($\alpha_{IIb}\beta_3$ integrin) leads to platelet aggregation. Tripeptide fragment—RGD (Arg-Gly-Asp) is responsible for this process.[2] Binding of fibrinogen to $\alpha_{IIb}\beta_3$ may be blocked by various RGD containing peptides. Consequently, RGD sequence has become a base for the design of new $\alpha_{IIb}\beta_3$ antagonists, platelet aggregation inhibitors. Antagonists of $\alpha_{IIb}\beta_3$ receptor are represented by monoclonal antibodies, RGDF and RGDS containing peptides, RGD mimetics.[3] RGDF mimetics receive a great interest from investigators as promising synthetic $\alpha_{IIb}\beta_3$ antagonists which may be obtained relatively easily.

It is considered[4] that RGDF mimetics should incorporate both basic and acidic binding centers for the effective interaction with $\alpha_{IIb}\beta_3$ receptor. The presence of hydrophobic fragment in the linker which connects these basic and acidic centers positively influences on the antiaggregative properties of RGDF mimetics.[4] The compounds containing 2*H*-1,4-benzoxazine-3(4*H*)-one scaffold were synthesized by the authors[5,6] and found to be potent $\alpha_{IIb}\beta_3$ antagonists. The distance between basic moiety (guanidino-, amidino-, amino-, etc.) and carboxylic function in molecules of potent RGDF mimetics is of 10–15 Å.[4,7] The structure of peptidomimetic **1** developed by the authors[7] through modification of cyclopeptide **2** (Fig. 1) corresponds to all these requirements. Insertion of the residue of *m*-aminobenzoic acid makes a mimetic molecule fairly rigid and improves its activity. The IC_{50} value of antiaggregative activity for the compound **1** is 200 nM.[7]

(Aminobenzamidino)succinyl was proposed as Arg-Gly surrogate for obtaining RGDF mimetics (ABAS series).[8] The linear mimetics **3** and **4** based on the residues of 4-(isoindoline-5-yl)amino-4-oxobutyric[9] and 4-(1,2,3,4-tetrahydroisoquinoline-7-yl-amino)-4-oxobutyric[10] acids as Arg-Gly fragment surrogates were synthesized by us earlier (Fig. 2). Asp-Phe motif was replaced by the residues of β-alanine and D,L-β-phenyl-β-alanine. Obtained mimetics have demonstrated a high in vitro antiaggregative activity and a high affinity for $\alpha_{IIb}\beta_3$ in suspension of washed human platelets.[9,10]

Study in this paper concerns the possibility to use the residue of 3-[(1,2,3,4-tetrahydroisoquinoline-7-yl-amino)carbonyl]benzoic acid as Arg-Gly surrogate for RGDF mimetic creation. β-Alanines containing various substituents in β-position are successfully used for the

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* Corresponding author. Tel.: +38 0482 66 30 41; fax: +38 0482 65 20 12; e-mail: peptides@paco.net

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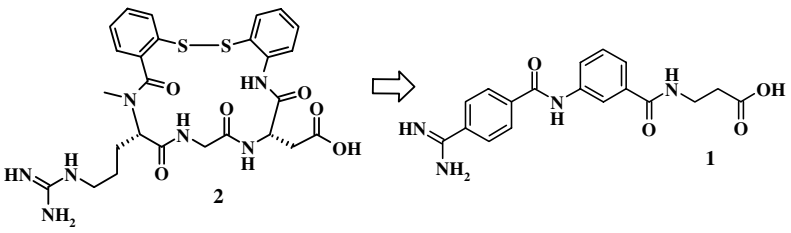


Figure 1. Structures of mimetic **1** and cyclopeptide **2** (SK&F 106760).

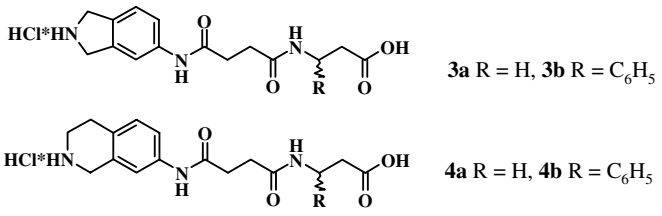
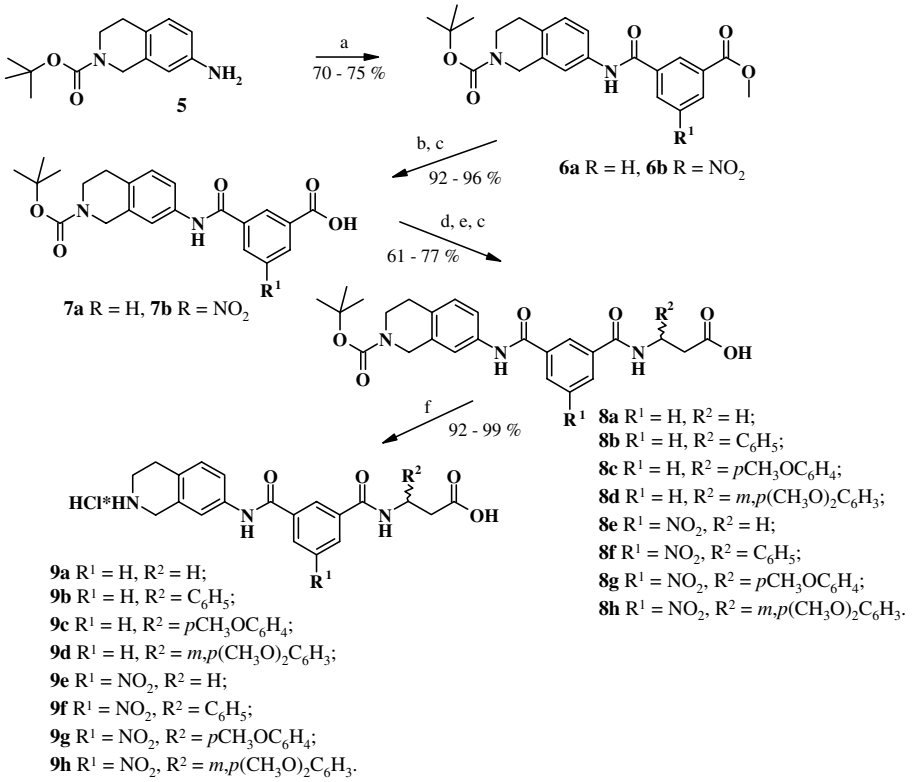


Figure 2. Structures of mimetics **3** and **4**.

synthesis of RGDF mimetics, fibrinogen receptor blockers. The residue of β-substituted β-alanine is considered to mimic Asp-Phe motif.[4,11] Aromatic substituent gives a possibility for additional binding of a ligand to the $\alpha_{IIb}\beta_3$, and carboxylic group of β-alanine imitates the side chain of aspartic acid residue.

7-Amino-2-Boc-1,2,3,4-tetrahydroisoquinoline[10] (**5**) was used as a initial compound (Scheme 1). Acylation of the compound **5** by chloroanhydrides of monomethyl esters of isophthalic or 5-nitroisophthalic acids has given derivatives **6a** and **6b**, and further saponification of their ester groups has afforded corresponding acids **7a** and **7b**. The compounds **8** were obtained by condensation of the acids **7a** and **7b** with β-alanines' sodium salts conducted by DCC method with SuOH addition. Removal of Boc-groups of intermediate compounds **8** afforded target RGDF mimetics **9**. All β-substituted β-alanines used in syntheses represented racemic mixtures.



Scheme 1. Reagents and conditions: (a) chloroanhydrides of monomethyl esters of isophthalic or 5-nitroisophthalic acids, Et_3N, chloroform, 0 °C; (b) 1 M NaOH, MeOH/H_2O, 20 °C; (c) 1 M HCl, H_2O, 0 °C; (d) DCC, SuOH, THF, 0 °C; (e) β-amino acids, $NaHCO_3$, H_2O, 0 °C; (f) 4 M HCl in dioxane, 0 °C.

Table 1. Biological properties of RGDF mimetics **3a**, **3b**, **4a**, **4b** and **9a**–**9h**

Compound	Antiaggregative activity, in vitro assays on human PRP, IC_{50}[a] (nM)	Inhibition of FITC-Fg binding to $\alpha_{IIb}\beta_3$ on the surface of human activated platelets, IC_{50}[a] (nM)
3a	2750.0 (±370.0)[9]	14.0 (±2.1)[9]
3b	860.0 (±120.0)[9]	8.3 (±1.4)[9]
4a	30.0 (±1.6)[10]	1.2 (±0.14)[10]
4b	13.0 (±1.0)[10]	1.0 (±0.12)[10]
9a	78.0 (±6.5)	9.0 (±0.56)
9b	25.0 (±1.7)	1.0 (±0.41)
9c	17.0 (±1.6)	0.8 (±0.07)
9d	11.6 (±1.3)	0.7 (±0.07)
9e	2300.0 (±160.0)	32.0 (±2.2)
9f	710.0 (±39.0)	8.0 (±0.6)
9g	450.0 (±41.0)	5.0 (±0.4)
9h	340.0 (±23.0)	3.7 (±0.4)

[a] Values are means of three experiments, standard deviation is given in parentheses.

The compounds **9** have demonstrated a high in vitro antiaggregative activity in bioassays on a human platelet-rich plasma (PRP) (Table 1) by Born's method[12,13] in blood obtained from at least three donors. Platelet aggregation was induced by ADP. In order to reveal the molecular mechanism of antiaggregatory action of RGDF mimetics **9**, their influence on specific binding of fluoresceinisothiocyanate-labeled fibrinogen (FITC-Fg) to $\alpha_{IIb}\beta_3$ (in a suspension of human washed platelets) was examined by the procedure.[14,15] FITC-Fg obtained by the method[16] specifically bound to platelet receptors with a dissociation constant (K_d) of 1.02 μM. Experimental data (Table 1) evidently show high affinities of the compounds **9** for $\alpha_{IIb}\beta_3$.

Earlier, it was demonstrated by us that incorporation of phenyl in the β-position of β-alanine residue in the compound **3a** brought a 3-fold increase in antiaggregative activity for the compound **3b** and a 2-fold increase in its affinity.[9] For the derivatives of 4-(1,2,3,4-tetrahydroisoquinoline-7-yl-amino)-4-oxobutyric acid, this structure modification, practically, produced no effect on the affinity of mimetic **4b** for $\alpha_{IIb}\beta_3$, while its antiaggregative activity had enhanced almost twice corresponding to the compound **4a**.[10] Similar tendency could be traced also for the peers mimetics **9a** and **9b**, **9e** and **9f**. In general, biological activity of the compounds containing fragment of isophthalic acid (mimetics **9a** and **9b**) was lower than that of their analogs **4a** and **4b**. The presence of methoxy substituents in *para* position of the phenyl groups situated in β-alanine moieties of RGDF mimetics **9c** and **9g** afforded a minor enhancement of indexes for inhibition of FITC-Fg binding to $\alpha_{IIb}\beta_3$ and antiaggregative activity of these compounds relative to their analogs **9b** and **9f**, respectively. Introduction of second methoxy group in the *meta* position of benzene ring also insignificantly improved values of IC_{50} both for binding to $\alpha_{IIb}\beta_3$ and for platelet aggregation inhibition in in vitro assays for the compounds **9d** and **9h**, compared to their analogs **9c** and **9g**. Comparison of biological properties of the mimetics **9a**–**9d** without nitro group in isophthalic acid fragment with those of their analogs **9e**–**9h** containing nitro groups makes it possible to note negative influence of a nitro group on inhibition of FITC-Fg binding to $\alpha_{IIb}\beta_3$ and antiaggregative activity. RGDF mimetics **9c** and **9d** have demonstrated maximum affinity for $\alpha_{IIb}\beta_3$ receptors on the surface of human washed platelets.

Experimental data obtained on antiaggregative activity and inhibition of FITC-Fg binding to fibrinogen receptor allow to consider the novel RGDF mimetics based on 3-[(1,2,3,4-tetrahydroisoquinoline-7-yl-amino)carbonyl]benzoic acid and β-substituted β-alanines as potent platelet aggregation inhibitors and $\alpha_{IIb}\beta_3$ receptor antagonists.

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